

AMENDMENTS TO THE CLAIMS:

Claim 1 (currently amended) A process for the preparation of cross linked enzyme crystals of hydrolases, and oxidoreductases which are solvent tolerant, thermostable and shear resistant, the process comprising the steps of :

(a) crystallizing ~~the enzymes~~ an enzyme in aqueous buffer with a suitable salts and ~~co-solvents~~ a co-solvent in the presence of surfactants at a temperature ranging ~~between~~ from about 4° to about 10° C for a period ranging ~~between~~ from about 5 hr. to about 15 days to obtain ~~the enzyme~~ crystals ~~of the protein~~ having a particle size ranging ~~between~~ from about 50 to about 150 microns;

(b) reacting the enzyme crystals ~~of the enzyme~~ obtained in step (a) with a multifunctional crosslinking agent in the presence of buffer of pH ranging ~~between 3-8~~ from about 3 to about 8 at a temperature ranging ~~between~~ from about 4° to about 25° C to get ~~the~~ crossed linked enzyme crystal crystals;

(c) washing the cross linked crystals with a reagent that is capable of removing the excess of the said multifunctional cross linking ~~reagent~~ agent so as to obtain ~~the~~ washed cross linked protein crystals; and

(d) coating the cross linked protein crystals with a suitable surfactant, and lyophilizing ~~it~~ the protein crystals to obtain ~~the~~ a stable product.

2. (currently amended) The process as claimed in claim 1, wherein ~~said enzymes~~ the enzyme is selected from the group consisting of hydrolases and the said enzyme ~~is~~ comprises a starch hydrolyzing amylase namely glucoamylase.

3. (currently amended) A process as claimed in claim 1, wherein said oxidoreductase enzyme ~~is~~ comprises a plant peroxidase.

4. (currently amended) The process as claimed in ~~claims 1 to 3~~ wherein said ~~oxidase~~ oxidoreductase is selected from the group of plant peroxidases consisting of Horse radish, Ipomea or Saccharum peroxidases.

5. (currently amended) A process as claimed in claim 1 wherein the crystallizing salt is comprises a salt selected from the group consisting of sulphate of ammonium or and sulphate of sodium either as saturated solution or crystals.

6. (currently amended) A process as claimed in claim ~~1~~ 2 wherein the said buffer used for the cross linked glucoamylase preparation is an aqueous buffer of 10mM -0.5M of acetate having a pH of about 4.5.

7. (currently amended) A process as claimed in claim ~~1~~ 3 wherein the said buffer used for the cross linked peroxidase preparation is an aqueous buffer of 10mM -0.5M phosphate or tris having a pH of about 6.5-8.0.

8. (currently amended) A process for the preparation of the cross linked ~~protein~~ enzyme ~~crystal~~ crystals as claimed in claim 1, wherein the said co-solvent ~~is~~ an alcohol having a concentration of about 1 to about 20% -20%, example selected from the group consisting of 2-methyl-2,4-pentane diol; 2-methyl-2,4-pentane diol, 2-propanol; 1,5-pentane diol, 1,5-pentane diol, ethanol, methanol, and isoamyl alcohol.

9. (currently amended) A process as claimed in claim ~~claims 1 to 8~~, wherein said ~~crystal is a microcrystal~~ crystals are microcrystals of about 150 microns or less.

10. (currently amended) A process as claimed in claim 1, wherein the cross linking ~~reagents~~ agent ~~used is~~ comprises glutaraldehyde, and starch dialdehyde.

11. (currently amended) A process as claimed in claim 1, wherein the said surfactant ~~used is~~ is selected from the group consisting of anionic, non-ionic, ~~or~~ and cationic surfactants.

12. (currently amended) A process as claimed in claim ~~claims 1 to~~ 11 wherein the ~~cationic~~ surfactant comprises a cationic surfactant selected from the group consisting of ~~used is~~ cetyl trimethyl ammonium bromide ~~or~~ and cetrimide.

13. (currently amended) A process as claimed in claim 11 ~~claims 1-12~~ wherein the ~~anionic~~ surfactant ~~used is~~ is an anionic surfactant comprising dioctylsulfosuccinate Aerosol OT.

14. (currently amended) A process as claimed in ~~claims 1 to 13~~ claim 11 wherein the ~~non-ionic~~ surfactant is a non-ionic surfactant ~~used is~~ selected from the group consisting of alkyl phenol ethoxylate, sorbitan trioleate, sorbitan tristerate. ~~Examples Tween 20, Tween 80 and Triton X-100.~~

15. (currently amended) A process as claimed in claim 1 ~~claims 1 to 14~~ wherein the said surfactant provides a weight ratio of ~~crosslinked enzyme~~ cross linked protein crystals to surfactant between about 1:1, and about 1:5, ~~preferably between about 1:1 and about 1:2~~ and is in a lyophilized form.

16. (currently amended) The process as claimed in claim 2 ~~4~~, wherein the cross linked gulcoamylase is active in a 1:1 mixture of water organic solvents n-dodecane; n-hexane; chloroform; and dimethyl sulphoxide.

17. (currently amended) A process as claimed in claim 1 ~~any of the preceding claims~~, wherein the said ~~crosslinked~~ cross linked enzyme ~~crystal is having~~ crystals have resistance to exogenous proteolysis, such that said ~~crosslinked~~ cross linked enzyme ~~crystal retains~~ crystals retain at least 91% of ~~its~~ their initial activity after incubation for three hours in the presence of a concentration of Protease that causes the soluble uncrosslinked form of the enzyme that is crystallized to form said enzyme ~~crystal that is~~ crystals that are crosslinked to lose at least 94% of ~~its~~ their initial activity under the same conditions, wherein said crystals are ~~crystal is~~ in lyophilized form.

18. (currently amended) The process as claimed in claim 3 ~~4~~, wherein the cross linked Peroxidases are active in organic solvents ~~like~~ selected from the group consisting of toluene; 80% dioxane, chloroform; 2-propanol; chloroform; acetone; ethanol; acetonitrile; methnol; and dioxane.

19. (currently amended) A process of continuous generation of glucose solution making use of the cross linked enzyme ~~crystal~~ crystals as claimed in claim 2 ~~claims 1 to 18~~, wherein the said cross linked glucoamylase crystals are packed in a jacketed column for the continuous saccharification of starch solution having a concentration of about 1 to about 20 ~~1-20%~~ preferably 4-10% (W/V) at a pH of about 4.5 and at about 60° C with a yield of about 110g glucose /L/hour at a residence time of about 7.6 min.

20. (currently amended) A process of continuous generation of glucose solution making use of the cross linked glucoamylase ~~crystal~~ crystals as claimed in claim 19, wherein the said enzyme crystals ~~can also~~ act upon a solution of about 1 to about 30 % ~~1-30%~~ (W/V) of maltodextrin of DE 10-15 preferably 10% (W/V) ~~maltodextrin with a DE of 10-14~~ at a pH of about 4.5, at about 60° C thereby producing a glucose solution within about 1-8 min with a yield of about 463 to about 714 g/L/h.

21. (currently amended) A process as claimed in claim 4 ~~claims 1 to 18~~ wherein the crystals of plant peroxidase ~~especially comprising~~ Horse radish peroxidase produce 2,4-dimethyl ~~produces 2,4-dimethyl~~ phenol dimer ~~dimmer~~ from monomer dissolved either in 2-propanol or toluene and the catalysis is carried out at about 50° C for about 30 min. in the presence of about 30% H₂O₂.

22. (new) A process as claimed in claim 1 wherein the said surfactant provides a weight ratio of cross linked protein crystals to surfactant between about 1:1 and about 1:2 and is in a lyophilized form.

23. (new) A process of continuous generation of glucose solution making use of the cross linked enzyme crystals as claimed in claim 2, wherein the said cross linked glucoamylase crystals are packed in a jacketed column for the continuous saccharification of starch solution having a concentration of about 4 to about 10% (W/V) at a pH of about 4.5 and at about 60° C with a yield of about 110g glucose/L/hour at a residence time of about 7.6 min.

24. (new) A process of continuous generation of glucose solution making use of the cross linked glucoamylase crystals as claimed in claim 19, wherein the said enzyme crystals act upon a solution of about 10%(W/V) of maltodextrin of DE 10-14 at a pH of about 4.5, at about 60° C thereby producing a glucose solution within about 1 to about 8 min with a yield of about 463 to about 714 g/L/h.